AN APPRAISAL OF RAPD-PCR: A NEW MOLECULAR TOOL FOR THE IDENTIFICATION OF INVERTEBRATE SPECIES

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The use of DNA probes to identify species, in particular the RAPD-PCR (random amplified polymorphic DNA by polymerase chain reaction) technique, has become widespread in microbiology, and is rapidly gaining acceptance in plants. Bands that remain constant in all individuals of a population or species can be used for identification of those groups. Once suitable primers have been determined for each group of species, large numbers of samples can be analysed rapidly and inexpensively. A major advantage of this technology is the ability to identify species from any stage in the life cycle. □ *DNA, PCR, molecular, identification, invertebrates.*

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The use of DNA probes to identify species has become widespread in microbiology, and is rapidly gaining acceptance in plants. In particular, the relatively quick and inexpensive RAPD-PCR (random amplified polymorphic DNA by polymerase chain reaction) technique has been used for species analysis and identification in many commercial cultivars, and wild species (Hu & Quiron, 1991; Chalmers et al., 1992).

Black et al. (1992) have recently amplified insect DNA using RAPD-PCR. They found species-specific and biotype-specific banding patterns in adult and nymph aphids. The technique also detected parasite and host species in parasitised aphids. Mention was made of having used the technique in hessian flies (Diptera: Cecidomyiidae), deltocephaline leafhoppers (Hemiptera: Cicadellidae), tetranychid mites (Acari: Tetranychidae) and aedine mosquitoes (Diptera: Cecidomyiidae), but only the latter case has the work been published (Kambhampati et al., 1992; Ballinger-Crabtree et al., 1992).

The polymerase chain reaction (PCR) is now one of the most widely used tools in molecular biology. Specific regions of the genome can be amplified in vitro using PCR. Two oligonucleotide primers (flanking the region to be amplified) are used to direct enzymatic synthesis of the specified region of the genomic DNA which acts as a template. When specific primers are used, a single gene can be amplified from minute amounts of target DNA to the extent that it can be visualised under ultra violet light on agarose gels, after staining with ethidium bromide. RAPD-PCR involves the use of slightly less-specific primers that hybridise to and amplify multiple arbitrary regions of the genome (Williams et al., 1990). When this amplified DNA is separated according to size by agarose gel electrophoresis, variation of the resulting pattern of bands between individuals and populations represents genetic polymorphisms. These can be used for parentage assays and to investigate genetic variation within and between populations (Caetano-Anolles et al., 1991; Hadrys et al., 1992). Allelic variation can result in the loss of a band. This may limit genetic analysis, particularly of hybrids; but should be overcome by the ability to analyse multiple alleles. Bands that remain constant in all individuals of a population or species can be used for identification of those groups. For this application, it is not necessary to know what genes are amplified, we only require enough constant bands to distinguish species etc. In the gels of Black et al. (1992) there were typically three to six constant bands for each species. With at least 30 possible band positions distinguishable on these gels, (and up to 100 bands on silver stained polyacrylamide gels, Caetano-Anolles, et al., 1991), thousands of combinations would allow plenty of scope for species diagnostics.

The most time consuming aspect of getting the technique up and running is determining the most suitable primers for a particular group of taxa. Most of the RAPD-PCR primers trialed by Black et al. (1992) gave species specific banding patterns for the majority of species tested. It is only a matter of time before they and other workers publish sequences of primers suitable for a ranges of other taxa. Some of the primers used by Black

et al. (1992) were designed by Operon Technologies Inc., California; primers may already be available and/or being developed commercially.

Once suitable primers have been determined for each group of species, large numbers of samples can be analysed rapidly (results within 24 hours) and inexpensively (less than $10 per sample including labour of about 2.5 hours for a batch of 20 samples) with existing molecular laboratory facilities. (A basic laboratory for these assays could be equipped for less than $20,000).

Although PCR can be accomplished from a single cell, in practice this is difficult, and samples of tissue at least visible to the naked eye are preferable. The technique involves crushing specimens in an extraction buffer which contains detergent to solubilise macromolecules, and proteinase K to digest proteins. After 3 minutes at 95°C, the samples can be used directly for PCR, or frozen until needed (Black et al., 1992). The PCR is left to run for 12 hours (usually overnight) and the products are analysed by standard agarose gel electrophoresis. Any specimen or tissue containing nucleated cells can be used.

A major advantage of this technology is the ability to identify species from any stage in the life cycle (eg. larvae pupae, eggs etc.). The DNA sequence (and therefore the RAPD-PCR band pattern) remains unchanged throughout the life cycle, and also for all types of tissues. This will be particularly useful when morphological characteristics of particular stages make species identification difficult (eg. freshwater forms). Unknown nymphs etc. can be easily identified if they have RAPD-PCR banding patterns consistent with those of known adult specimens.

Keys based on RAPD-PCR patterns could be developed for species identification, and this process could be readily computerised. Combined with automated gel reading [eg. using Applied Biosystems Model 373A DNA Sequencing System to give computerised output of data from gels prepared from RAPD-PCR using fluorescent labelled primers (Applied Biosystems, 1992)] RAPD-PCR promises to be a major tool for species identification.

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